DETECTION OF RUPTURE EVENT SENSORS

This invention relates to apparatus for separation and detection of analytes and for measuring the affinity between analytes or binding partners using Rupture Event Scanning (REVS™). In particular, it relates to apparatus for exciting mechanical oscillations in a resonator and sensing and analysing signals produced in the resonator in response to a rupture event.

The use of a thickness shear mode resonator (TSMR) for determining the affinity between binding partners is known. WO01/02857 describes a method and apparatus using a TSMR, such as is used in a quartz crystal microbalance (QCM), for this purpose. The apparatus causes the crystal to oscillate and increases the amplitude of oscillation until the acceleration of a target molecule attached to a surface of the crystal is sufficient to overcome the attractive force between the target molecule and its binding partner and cause the bond to be broken. When this rupture event occurs, the energy released can be detected in the form of further motional oscillations in the crystal. Such oscillations have been described as acoustic emission, but in reality constitute a complex set of oscillations of the crystal.

As described in WO01/02857, the crystal is caused to oscillate at its fundamental resonant frequency. Motional energy of the attached species is dissipated as the amplitude of the oscillation is increased, leading up to a release of significant energy at the rupture event. The electrical signal generated from the motional oscillations in the crystal is filtered near the third harmonic of the crystal driving frequency. The apparatus comprises an oscillator circuit for exciting the fundamental resonance of the crystal and a second frequency synthesizer connected to an analyser to which the filtered output from the crystal is also connected. The second frequency synthesizer operates at a frequency, Δf, higher than the

third harmonic of the driving frequency such that, when it is mixed by the analyser with the third overtone output from the crystal, a signal at the difference frequency, Δf, is produced. Alternatively, the crystal may be excited near an overtone resonance of the crystal and the motional oscillations detected at the fundamental frequency.

This system has the disadvantage that an external frequency synthesizer of high precision and very low noise is required and this increases the complexity of the method and apparatus as well as rendering the apparatus heavier, larger and more expensive.

In accordance with the present invention, there is provided apparatus for separating an analyte from a mixture or for detecting an analyte or for determining the affinity, or a property related to affinity, between binding partners comprising:

a) a surface having the analyte or one of the binding partners immobilised thereon, in use;

b) a transducer for oscillating the surface;

c) a controller connected to the transducer for varying the amplitude and/or frequency of the oscillation to cause a dissociation event; and,

d) an analyser connected to the transducer for detecting an oscillation of the transducer due to the dissociation event;
characterised in that the controller includes an oscillator connected in a resonant circuit with the transducer such that the transducer oscillates at two frequencies simultaneously, one of these causing the transducer to oscillate the surface and the other being supplied as an output to the analyser.

In accordance with a second aspect of the present invention, there is provided a method for separating an analyte from a mixture or for detecting an analyte or for determining the affinity, or a property related to affinity, between binding partners, the method comprising:

a) immobilising the analyte or one of the binding partners on a surface;

b) oscillating the surface;

c) varying the amplitude and/or frequency of the
5   oscillation to cause a dissociation event; and,

d) detecting an oscillation due to the dissociation event using an analyser;

characterised by oscillating the surface at two frequencies simultaneously, one of these causing the surface to
10  oscillate and the other being supplied as an output to the analyser for use in detecting the oscillation due to the dissociation event.

Hence, the invention provides an apparatus for separating an analyte from a mixture, detecting an analyte
15  or for determining the affinity, or a property related to affinity, between binding partners that is simpler, cheaper and more portable than the prior art.

The transducer may oscillate at any two frequencies but, in one embodiment, the frequency that is supplied as
20  an output to the analyser is a multiple of the frequency that causes the surface to oscillate. For example, the frequency that causes the surface to oscillate is the transducer's fundamental resonant frequency and the frequency supplied as an output to the analyser is one of
25  the transducer's overtone frequencies.

In an alternative embodiment, the frequency that causes the surface to oscillate is a multiple of the frequency that is supplied as an output to the analyser. For example, the frequency that causes the surface to
30  oscillate is one of the transducer's overtone frequencies and the frequency supplied as an output to the analyser is the transducer's fundamental frequency.

Typically, the oscillation of the transducer due to the dissociation event is at a range of frequencies located
35  around at least one of the transducer's resonant frequencies.

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The apparatus may be used with a variety of analytes and binding partners but typically, the immobilised analyte or binding partner is a metal (for example, gold, silver, copper or aluminium), a polymer (for example, polystyrene,

5    poly vinyl alcohol or a polysaccharide), a dendrimer, a self-assembled monolayer, a peptide, a protein, an antibody, an antigen, an enzyme, enzyme inhibitor, a biologically active molecule, a drug, a polynucleotide (for example DNA) or a peptide-polynucleotide (for example

10   PNA). Alternatively, the immobilised analyte or binding partner may be a biological entity such as a cell, a bacterium, a virus, a prion, an amyloid, a proteinaceous aggregate or a phage.

The apparatus may be used with only one analyte or

15   pair of binding partners, one of which is immobilised on the surface. However, the apparatus may be used with several analytes or binding partners simultaneously. Thus, typically, different analytes or binding partners are immobilised at different positions on the surface.

20   Normally, the dissociation event is detected as a motional oscillation. This motional oscillation may produce an electrical signal in the transducer.

Typically, the transducer is an acoustic wave device, and in this case it may be a piezoelectric transducer, such

25   as a quartz crystal, as used in a microbalance, or a surface acoustic wave device. A suitable piezoelectric transducer may comprise zinc oxide, a piezoelectric polymer (for example polyvinylidene fluoride), a piezo-electric semiconductor (for example Gallium Arsenide) or a

30   piezo-ceramic (for example lithium tantalate). Alternatively, the transducer may be an electromagnetic device.

When the transducer is a piezoelectric transducer, the oscillator may be a dual mode crystal resonator.

35   In a preferred embodiment, the oscillator comprises two bandpass filters, each having its input connected to the transducer and its output connected to a respective

amplifier, the outputs of which are combined by a power
adder and supplied to the transducer, the centre
frequencies of the bandpass filters corresponding to the
two oscillating frequencies of the transducer.

5        Typically, the analyser comprises a radiofrequency
detector and a digitiser.

An example of the invention will now be described with
reference to the accompanying drawings, in which:-

Figure 1 shows, schematically, one embodiment of the
10   invention;

Figures 2 and 3 each show, schematically, a first
implementation of the embodiment of Figure 1;

Figure 4 shows, schematically, a second implementation
of the embodiment of Figure 1;

15       Figure 5 shows, schematically, a third implementation
of the embodiment of Figure 1;

Figure 6 shows an example of a power adder for use in
the first to third implementations;

Figure 7 shows an example of a radiofrequency detector
20   and AGC regulator; and,

Figure 8 shows, schematically, a fourth implementation
of the embodiment of Figure 1.

More specifically, Figure 1 shows a thickness shear
mode resonant sensor such as a quartz crystal 1 connected
25   in a resonant circuit with oscillator 2. Oscillator 2 is
a dual frequency crystal oscillator and excites two
resonant oscillations of crystal 1, namely the fundamental
and its third overtone.

An output from oscillator 2 is connected to signal
30   processor 3. The output from signal processor 3 is
connected to computer 4 which analyses and displays the
results. Computer 4 also has a control output connected to
oscillator 2 for varying the amplitude of oscillation.

In use, one of a pair of binding partners for which it
35   is wished to determine the affinity is immobilised on the
surface of the crystal 1, which is caused to oscillate at
its fundamental and third overtone frequencies by

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oscillator 2. The magnitude of the third overtone
oscillation of crystal 1 is much lower in magnitude than
that of the fundamental oscillation such that it is
comparable in amplitude with the signal produced when a
5    rupture event occurs.

It is important to note that the energy released by
the rupture event has been found to contain many frequency
components. In particular these can be found in bandwidths,
which include a harmonic of the driving frequency, and/or
10   a resonant overtone frequency of the crystal. For the
third harmonic and overtone, this bandwidth may be in the
region of 200kHz, but will vary depending on the harmonic
used, and the properties of the crystal. It is not easy to
detect the energy release by measuring the signal from the
15   transducer exactly at the third harmonic of the driving
signal because the driving signal applied to the transducer
also causes oscillations of the transducer at its
harmonics, due to the non-linear response of the
transducer. These harmonic oscillations have a very narrow
20   bandwidth, but in intensity mask the oscillations induced
at close frequencies by the rupture event. The detection
frequency is therefore best offset from the third harmonic
of the fundamental resonance frequency (i.e. it is selected
to be at a frequency, $\Delta f$ above or below the third
25   harmonic), and the bandwidth of the detection system is
chosen following experimental evaluation to maximise the
signal to noise ratio. For example, an offset of 70-80kHz
from the third overtone resonance of the crystal with a
bandwidth of up to 20kHz has been found to give a good
30   signal to noise ratio. Those skilled in the art will
recognise that many variations are possible.

Computer 4 provides a control signal to oscillator 2
for varying the amplitude of oscillation of crystal 1. The
amplitude of oscillation is increased, thereby increasing
35   the acceleration of a binding partner immobilised on the
surface of crystal 1 until the attractive force between the
immobilised binding partner and another binding partner

atached to it is overcome. When this occurs, the binding
partners separate from each other, and this rupture event
is detected by crystal 1 which produces an electrical
signal in response to the associated motional oscillations.

5       Alternatively, an analyte may be immobilised on the
surface of the crystal 1. The amplitude of oscillation is
again increased until the attractive force between the
analyte and the surface is overcome. The rupture event is
detected as already described.

10      In an alternative embodiment, computer 4 varies the
frequency of the driving oscillation of oscillator 2, since
variation of the oscillation frequency can also be used to
control the acceleration of crystal 1.

        The energy released by the rupture event causes
15  crystal 1 to oscillate, particularly near its resonant
frequencies. This typically provides a signal centred
around the third resonance overtone. Signals at other
frequencies are also produced. The signal centred around
the third overtone is isolated by signal processor 3 and
20  displayed, after suitable analysis, on computer 4.

        A first implementation of the embodiment described
with reference to Figure 1 is shown in Figure 2. In this,
oscillator 2 comprises bandpass filters 10a,10b, variable
gain amplifiers 11a,11b and power adder 13. As such,
25  bandpass filters 10a,10b, variable gain amplifiers 11a,11b,
power adder 13 and crystal 1 form a resonant loop. Bandpass
filter 10a has a centre frequency at the fundamental
frequency of crystal 1 whilst bandpass filter 10b has a
centre frequency at $\Delta f$ above the third harmonic of the
30  fundamental frequency. Variable gain amplifier 11a
amplifies the signal from bandpass filter 10a whilst
variable gain amplifier 11b amplifies the signal from
bandpass filter 10b. The outputs from variable gain
amplifiers 11a,11b are added together by power adder 13,
35  the output of which is connected to crystal 1.

        Power adder 13 combines the signals from variable gain
amplifiers 11a,11b in such a way that the signal from

variable gain amplifier 11a, which is at the fundamental
frequency of crystal 1, forms the majority (typically
99.9%) of the output of power adder 13 and the minority
part of the output signal from power adder 13 (typically
5    0.1%) is formed from the output of variable gain amplifier
11b. A suitable power adder is shown in Figure 6. This
comprises two resistors 100,101. The value of resistor 101
is typically significantly higher than that of resistor
100. In this example, resistor 101 has a value 1000 times
10   greater than that of resistor 100 such that the signal at
their junction comprises 99.9% of the signal supplying
resistor 100 and 0.1% of the signal supplying resistor 101.
In this way, crystal 1 oscillates at both its fundamental
frequency and at a frequency $\Delta f$ above the third harmonic.
15   The gains of variable gain amplifiers 11a,11b are
controlled by radio frequency (RF) detectors and automatic
gain control (AGC) regulators 12a,12b in response to
reference inputs 16a,16b. In this way, the gains of
variable gain amplifiers 11a,11b and hence, the magnitude
20   of oscillation of crystal 1 at each frequency of
oscillation can be independently controlled. The gain of
amplifier 11a is then increased until such time as a
rupture event occurs.

When a rupture event occurs the energy released causes
25   an additional oscillation of crystal 1 and the rupture
event signal that is produced is filtered by bandpass
filters 10a,10b. The output of variable gain amplifier 11b
is connected to RF detector and AGC regulator 16b which is
used to detect that portion of the rupture event signal
30   that was within the pass band of bandpass filter 10b. This
band pass can be chosen to select the range of frequencies
produced by the rupture event and to be detected by
detector 12b.

An example of a suitable circuit for use as RF
35   detectors and AGC regulators 12a,12b is shown in Figure 7.
This shows that the RF detector firstly squares an input
signal 102 using multiplier 103 and then low pass filters

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the resulting signal using resistor 104 and capacitor 105
to produce an output signal 106, which is proportional to
the power of the input. The proportional integral regulator
produces a time varying output which regulates the gain of
5     amplifiers 11a and 11b.

The squared output signal from RF detector and AGC
regulator 16b includes products of all frequency components
within the bandpass of filter 10b, and is time varying with
components at many frequencies. It is supplied to AC
10    amplifier and analogue-to-digital converter 14 which
samples this signal at a high frequency, and supplies a
digital version of its input signal to signal processing
and storage device 15. This provides buffering and reduces
the high data rate input to a data point set that can be
15    analysed on a PC. Together, RF detectors and AGC
regulators 12a,12b, AC amplifier and analogue-to-digital
converter 14 and signal processing and storage device 15
perform the role of signal processor 3 and computer 4.

A variant of the implementation of Figure 2 is shown
20    in Figure 3 in which the positions of bandpass filters 10a
and 10b have been swapped. The driving signal to crystal
1 is at the third overtone resonance of the crystal which
is separated from the third harmonic of the driving
frequency by the frequency difference $\Delta f$. The rupture event
25    signal is detected near the fundamental frequency of
crystal 1.

The implementation shown in Figure 3 has the advantage
that the excitation of the crystal 1 at an overtone
resonance does not induce subharmonics at the fundamental
30    frequency. In contrast, excitation at the fundamental
induces oscillations at overtone frequencies through
non-linearities in the transducer response. It is thus
easier to resolve the motional oscillation frequencies near
the fundamental whilst exciting the crystal 1 at an
35    overtone frequency than it is to resolve them at an
overtone frequency whilst exciting the crystal 1 at the
fundamental.

Figure 4 shows a second implementation of the invention which differs from the first implementation in that the RF detectors and AGC regulators 12a,12b are used only to control the gain of variable gain amplifiers 11a,11b and the AC amplifier and analogue-to-digital converter 14 is replaced by quadrature receiver 17 and analogue-to-digital converters 18.

Quadrature receiver 17 is supplied with the rupture event signal from bandpass filter 10b and a signal from frequency tripler 30 which is connected to the output of variable gain amplifier 11a and hence provides a signal at the third harmonic of the drive frequency. The quadrature receiver 17 produces two down converted output signals which are in-phase and in-quadrature components having a frequency that is the difference in frequency between the two inputs. If a rupture event occurs, it will produce quick modulation of the amplitude and phase of the frequencies passed by bandpass filter 10b which will then be transformed into time varying changes in the in-phase and in-quadrature amplitudes of the down converted signal output by quadrature receiver 17.

The in-phase and in-quadrature carriers are digitised by analogue-to-digital converters 18 and then these digital signals are supplied to signal processing and storage device 15 as before. In this implementation, RF detectors and AGC regulators 12a,12b, quadrature receiver 17, analogue-to-digital converters 18 , frequency tripler 30 and signal processing and storage device 15 perform the role of signal processor 3 and computer 4.

In the third implementation, shown in Figure 5, variable gain amplifier 11b is replaced by limiting amplifier 19b which is connected to bandpass filter 20b. Similarly, limiting amplifier 19a and bandpass filter 20a are connected between the output of bandpass filter 10a and variable gain amplifier 11a. The limiting amplifiers 19a, 19b have very high gains in the linear range and saturate at fixed positive and negative values of input voltage.

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This produces an output that is practically a square wave
of fixed amplitude. Limiting amplifiers 19a and 19b thus
control the amplitude of the signals instead of the
feedback loops through RF detectors and AGC regulators 12a

5    and 12b. Variable gain amplifier 11b as shown in Figures 2,
3 and 4 is therefore not required. Limiting amplifiers are
available as packages in integrated circuits and provide
advantages in terms of cost.

      Band pass filters 20a and 20b provide rejection of all

10   but the fundamental and the third overtone frequency
respectively, and the signal is a high quality sinusoid of
fixed amplitude. These filters must be of a very high
quality as they are required to attenuate the third
harmonic to a level significantly lower than the level of

15   the REVS signal. Thus, if the ratio of the signal levels
in the two branches of the power adder is 1000, the
attenuation must be at least 100,000 at the third
harmonic. In an alternative arrangement filter 20a can be
placed after power amplifier 21 to eliminate any harmonics

20   arising in power amplifier 21.

      In a further example of this type, it is also possible
to dispose of frequency tripler 30, and use the third
overtone signal directly as the reference to the quadrature
receiver, as this is the dominant oscillation frequency.

25         Another way of achieving self oscillation at two
frequencies simultaneously uses diplexers 80a,80b as shown
in Figure 8. These diplexers 80a,80b combine the function
of the bandpass filters and power adder shown in the
earlier figures. Use of diplexers 80a,80b has the

30   advantage that independent and continuous control of the
power levels supplied to the crystal at the fundamental and
third overtone frequencies can be achieved. Thus, the
amplitude of oscillation at the detection frequency can be
adjusted to an appropriate level to optimise the signal to

35   noise ratio. Either the fundamental frequency or third
overtone + $\Delta f$ signal can be used interchangeably as the
drive signal with the other frequency being used for

detection.    With the use of high isolation amplifiers (or variable attenuators) any loop can be inactivated and appear transparent to the other. Thus any number of frequencies can be provided for in the apparatus, and only
5    those required activated.        To increase oscillation stability and in order to remove any possible spurious modes a narrow band pass filter could be inserted directly after the outputs of each amplifier.